– the chromatogram obtained is similar to the chromatogram supplied with *clomifene citrate for performance test CRS*.

Limits:

– *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
– *impurities B, C, D, E, F, G, H*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
– *total*: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);
– *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak with a retention time relative to the clomifene peak of 0.2 or less.

(*Z*)-isomer. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25 mg of the substance to be examined in 25 mL of *0.1 M hydrochloric acid*, add 5 mL of *1 M sodium hydroxide* and shake with 3 quantities, each of 25 mL, of *ethanol-free chloroform R*. Wash the combined extracts with 10 mL of *water R*, dry over *anhydrous sodium sulfate R* and dilute to 100 mL with *ethanol-free chloroform R*. To 20 mL of this solution add 0.1 mL of *triethylamine R* and dilute to 100 mL with *hexane R*.

Reference solution. Dissolve 25 mg of *clomifene citrate CRS* in 25 mL of *0.1 M hydrochloric acid*, add 5 mL of *1 M sodium hydroxide* and shake with 3 quantities, each of 25 mL, of *ethanol-free chloroform R*. Wash the combined extracts with 10 mL of *water R*, dry over *anhydrous sodium sulfate R* and dilute to 100 mL with *ethanol-free chloroform R*. To 20 mL of this solution add 0.1 mL of *triethylamine R* and dilute to 100 mL with *hexane R*.

Column:

– *size*: *l* = 0.3 m, Ø = 4 mm;
– *stationary phase*: *silica gel for chromatography R* (10 µm).

Mobile phase: *triethylamine R, ethanol-free chloroform R, hexane R* (1:200:800 *V/V/V*).

Flow rate: 2 mL/min.

Detection: spectrophotometer at 302 nm.

Equilibration: with the mobile phase for about 2 h.

Injection: 50 µL.

Identification of peaks: the chromatogram obtained with the reference solution shows a peak due to the (*E*)-isomer just before a peak due to the (*Z*)-isomer.

System suitability: reference solution:

– *resolution*: minimum 1.0 between the peaks due to the (*E*)- and (*Z*)-isomers; if necessary, adjust the relative proportions of ethanol-free chloroform and hexane in the mobile phase.

Measure the area of the peak due to the (*Z*)-isomer in the chromatograms obtained with the test solution and the reference solution. Calculate the content of the (*Z*)-isomer, as a percentage of the total clomifene citrate present, from the declared content of *clomifene citrate CRS*.

Limit:

– *(Z)-isomer*: 30.0 per cent to 50.0 per cent.

Water (*2.5.12*): maximum 1.0 per cent, determined on 1.000 g.

ASSAY

Dissolve 0.500 g in 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

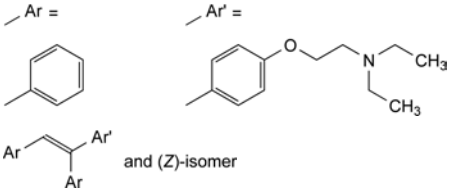
1 mL of *0.1 M perchloric acid* is equivalent to 59.81 mg of $C_{32}H_{36}ClNO_8$.

STORAGE

Protected from light.

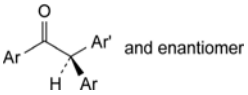
IMPURITIES

Specified impurities: *A, B, C, D, E, F, G, H.*

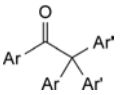


A. 2-[4-(1,2-diphenylethenyl)phenoxy]-*N,N*-diethylethanamine,

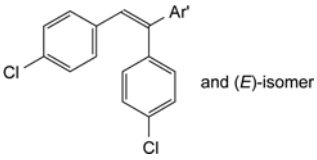
B. [4-[2-(diethylamino)ethoxy]phenyl]phenylmethanone,



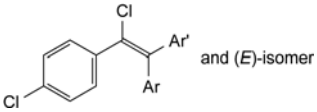
C. (2*RS*)-2-[4-[2-(diethylamino)ethoxy]phenyl]-1,2-diphenylethanone,



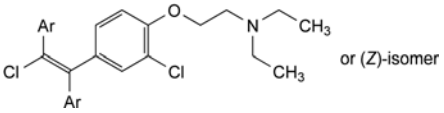
D. 2,2-bis[4-[2-(diethylamino)ethoxy]phenyl]-1,2-diphenylethanone,



E. 2-[4-[1,2-bis(4-chlorophenyl)ethenyl]phenoxy]-*N,N*-diethylethanamine,



F. 2-[4-[2-chloro-2-(4-chlorophenyl)-1-phenylethenyl]-phenoxy]-*N,N*-diethylethanamine,

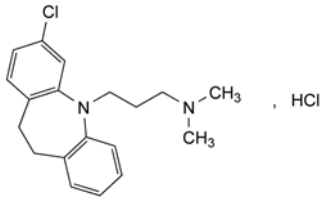


GH. 2-[2-chloro-4-(2-chloro-1,2-diphenylethenyl)phenoxy]-*N,N*-diethylethanamine (G. higher-melting-point isomer; H. lower-melting-point isomer).

01/2008:0889
corrected 6.0

CLOMIPRAMINE HYDROCHLORIDE

Clomipramini hydrochloridum



$C_{19}H_{24}Cl_2N_2$ M_r 351.3
[17321-77-6]

DEFINITION

3-(3-Chloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine hydrochloride.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or slightly yellow, crystalline powder, slightly hygroscopic.

Solubility: freely soluble in water and in methylene chloride, soluble in alcohol.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. Melting point (*2.2.14*): 191 °C to 195 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs of *potassium bromide R*. The transmittance at about 2000 cm^{-1} (5 µm) is at least 65 per cent without compensation.

Comparison: *clomipramine hydrochloride CRS*.

C. Thin-layer chromatography (*2.2.27*). *Prepare the solutions immediately before use and protected from light.*

Test solution. Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution. Dissolve 20 mg of *clomipramine hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate: *TLC silica gel G plate R*.

Mobile phase: *concentrated ammonia R, acetone R, ethyl acetate R* (5:25:75 *V/V/V*).

Application: 5 µL.

Development: over a path of 15 cm.

Drying: in air.

Detection: spray with a 5 g/L solution of *potassium dichromate R* in a 20 per cent *V/V* solution of *sulfuric acid R*. Examine immediately.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 5 mg in 2 mL of *nitric acid R*. An intense blue colour develops.

E. Dissolve about 50 mg in 5 mL of *water R* and add 1 mL of *dilute ammonia R1*. Mix, allow to stand for 5 min and filter. Acidify the filtrate with *dilute nitric acid R*. The solution gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S. Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 (*2.2.2, Method I*).

pH (*2.2.3*): 3.5 to 5.0 for solution S.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protected from light.*

Test solution. Dissolve 20.0 mg of the substance to be examined in a mixture of 25 volumes of mobile phase B and 75 volumes of mobile phase A and dilute to 10.0 mL with the same mixture of mobile phases.

Reference solution (a). Dissolve 22.6 mg of *imipramine hydrochloride CRS*, 4.0 mg of *clomipramine impurity C CRS*, 4.0 mg of *clomipramine impurity D CRS* and 2.0 mg of *clomipramine impurity F CRS* in a mixture of 25 volumes of mobile phase B and 75 volumes of mobile phase A and dilute to 100.0 mL with the same mixture of mobile phases. Dilute 1.0 mL of this solution to 10.0 mL with a mixture of 25 volumes of mobile phase B and 75 volumes of mobile phase A.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with a mixture of 25 volumes of mobile phase B and 75 volumes of mobile phase A.

Reference solution (c). Dissolve 10.0 mg of *clomipramine hydrochloride CRS* and 3.0 mg of *clomipramine impurity C CRS* in a mixture of 25 volumes of mobile phase B and 75 volumes of mobile phase A and dilute to 20.0 mL with the same mixture of mobile phases. Dilute 1.0 mL of this solution to 10.0 mL with a mixture of 25 volumes of mobile phase B and 75 volumes of mobile phase A.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *cyanopropylsilyl silica gel for chromatography R* (5 µm),
– *temperature*: 30 °C.

Mobile phase:

– *mobile phase A*: dissolve 1.2 g of *sodium dihydrogen phosphate R* in *water R*, add 1.1 mL of *nonylamine R*, adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 mL with *water R*,
– *mobile phase B*: *acetonitrile R*.

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	75	25
10 - 20	75 → 65	25 → 35
20 - 32	65	35
32 - 34	65 → 75	35 → 25
34 - 44	75	25

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL.

Relative retentions with reference to clomipramine (retention time = about 8 min): impurity A = about 0.5; impurity B = about 0.7; impurity C = about 0.9; impurity D = about 1.7; impurity E = about 2.5; impurity F = about 3.4; impurity G = about 4.3.

System suitability: reference solution (c):

– *resolution*: minimum 3.0 between the peaks due to clomipramine and to impurity C.

Limits:

– *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.0 per cent),
– *impurity C, D*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
– *impurity F*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
– *any other impurity*: not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
– *total of other impurities*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),
– *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),
– *disregard limit*: 0.01 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Heavy metals (*2.4.8*): maximum 20 ppm.

2.0 g complies with test C. Prepare the reference solution using 4 mL of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

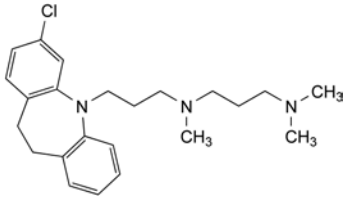
Dissolve 0.250 g in 50 mL of *alcohol R* and add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 35.13 mg of $C_{19}H_{24}Cl_2N_2$.

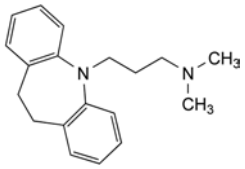
STORAGE

In an airtight container, protected from light.

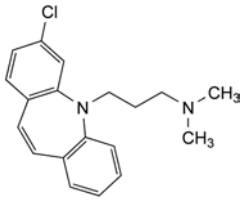
IMPURITIES



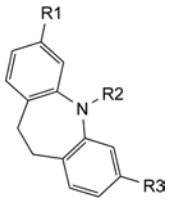
A. *N*-[3-(3-chloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)propyl]-*N,N′,N′*-trimethylpropane-1,3-diamine,



B. 3-(10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine (imipramine),



C. 3-(3-chloro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine,



D. R1 = R3 = Cl, R2 = CH_2-CH_2-CH_2-$N(CH_3)_2$: 3-(3,7-dichloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)-*N,N*-dimethylpropan-1-amine,

E. R1 = R2 = R3 = H: 10,11-dihydro-5*H*-dibenzo[*b,f*]azepine (iminodibenzyl),

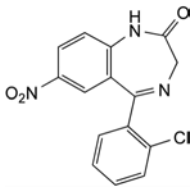
F. R1 = Cl, R2 = R3 = H: 3-chloro-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine,

G. R1 = Cl, R2 = CH_2-CH=CH_2, R3 = H: 3-chloro-5-(prop-2-enyl)-10,11-dihydro-5*H*-dibenzo[*b,f*]azepine.

01/2008:0890
corrected 6.0

CLONAZEPAM

Clonazepamum



$C_{15}H_{10}ClN_3O_3$ M_r 315.7
[1622-61-3]

DEFINITION

5-(2-Chlorophenyl)-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: slightly yellowish, crystalline powder.

Solubility: practically insoluble in water, slightly soluble in alcohol and in methanol.

mp: about 239 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *Ph. Eur. reference spectrum of clonazepam*.

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light and prepare the solutions immediately before use.*

Solvent mixture: *tetrahydrofuran R, methanol R, water R* (10:42:48 *V/V/V*).

Test solution. Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 20.0 mL with the same solvent. Dilute 1.0 mL to 10.0 mL with the solvent mixture.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve 5 mg of the substance to be examined and 5 mg of *flunitrazepam R* in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (c). Dissolve 1.0 mg of *clonazepam impurity B CRS* in the solvent mixture and dilute to 20.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm,
– *stationary phase*: *end-capped octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 10 volumes of *tetrahydrofuran R*, 42 volumes of *methanol R* and 48 volumes of a 6.6 g/L solution of *ammonium phosphate R* previously adjusted to pH 8.0 with a 40 g/L solution of *sodium hydroxide R* or *dilute phosphoric acid R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 10 µL.

Run time: 3 times the retention time of clonazepam.

Relative retention with reference to clonazepam (retention time = about 7 min): impurity B = about 2.1; impurity A = about 2.4.